TRANSPORT AND ION CHANNELS (MCB 507/8, 2003)

Biological membranes

Lipid bilayer
Hydrophobic interior and hydrohphilic outer surface

Importance of biological membrane

Contributes to cellular integrity
Help maintain intracellular concentration of solutes

Permeability properties of biological membranes (Fig. 1)

Impermeable to water molecules
Impermeable to ions and polar molecules
Permeable to gases and small uncharged polar molecules

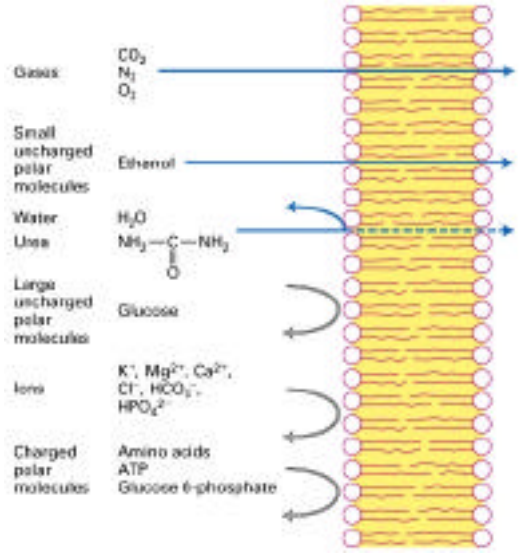

Fig. 1. Permeability through phospholipid bilayer

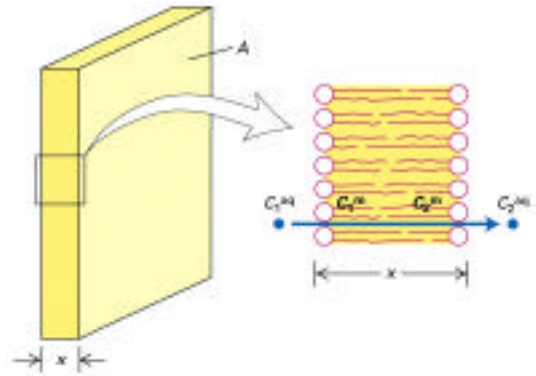

Fig. 2. A simple model for passive diffusion

Diffusion through lipid bilayer (Fig. 2)

Passive
No metabolic energy is expended
Movement is downhill (+ve ΔS or -ve ΔG)
Unaided by any transport protein
Little specificity to the process
Depends on hydrophobicity of the substance
Measured by partition coefficient (K),

Partition coefficient is a measure of the relative affinity for a substance for lipid versus water. K = equilibrium constant for its partition coefficient between oil and water

$K = C^m/C^{aq}$ (m = membrane; aq = aqueous)…… (1)

Higher the partition coefficient of the substance, the more lipid soluble it is. The first step in transport by diffusion is movement of the molecule from the aqueous phase into the hydrophobic interior of the lipid bilayer. Once the molecule is in the hydrophobic bilayer it diffuses across it. Finally, the molecule moves from the bilayer into the aqueous medium on the other side of the membrane. The interior of the lipid bilayer is typically 100-1000 times more viscous than water, therefore, the diffusion rate of a substance across the phospholipid bilayer is very much slower than the rate of diffusion of the same substance in water. Thus movement across the hydrophobic portion of the membrane is the rate-limiting step in the passive diffusion of molecules across cell membranes

Quantitative aspect of transmembrane diffuison

Membrane surface area = A
Thickness = x
Separates solutions of concentrations = C_1^{aq} and C_2^{aq} (where $C_1^{aq} > C_2^{aq}$)

Diffusion rate = dx/dt, is given by modification of Fick's Law, which states that the diffusion across the membrane is directly proportional to the permeability coefficient P, to the difference in solution concentrations $C_1^{aq} - C_2^{aq}$, and to the area A.

Therefore $dx/dt = PA(C_1^{aq} - C_2^{aq})$……... (2)

For any molecule, the value of P, and thus its rate of passive diffusion, is proportional to its partition coefficient K:

$P = KD/x$……. (3)

Where D = diffusion coefficient of the substance within the membrane and x is the membrane thickness. By substituting equations

We obtain $dx/dt = A.KD/x\ .(C_1^{aq} - C_2^{aq})$……(4)

Thus the rate of diffusion is proportional to both the partition coefficient and the diffusion constant and is inversely proportional to membrane thickness. The thickness of the hydrophobic interior of all phospholipid bilayer membrane is approximately the same, about 2.5 - 3 nm (25–30 Å), and the diffusion coefficient is the same for most substances. Therefore, for practical consideration, the differences in the rate at which molecules passively diffuse across membranes depend largely on their partition coefficients.

Functions of transport proteins:

Ingestion of essential nutrients
Excretion of metabolic waste products
Regulation of intracellular ion concentration

Protein-mediated transport could be of two major types (Fig. 3)

Passive transport (or facilitated diffusion): Down the concentration gradient.
Mediated by Uniporters and Channels

Active transport: Against the concentration gradient
Mediated by Primary pumps (eg. ATP-powered pump) and Secondary carriers (eg. Symporters and Antiporters)

PASSIVE TRANSPORT

Uniporter

Uniport (facilitated diffusion) versus passive diffusion (Fig. 4)

1) The rate of facilitated diffusion by uniporters is higher than that predicted by Fick's equation describing passive diffusion. Because the transported molecules never enter the hydrophobic core of the phospholipid bilayer, the partition coefficient K is irrelevant

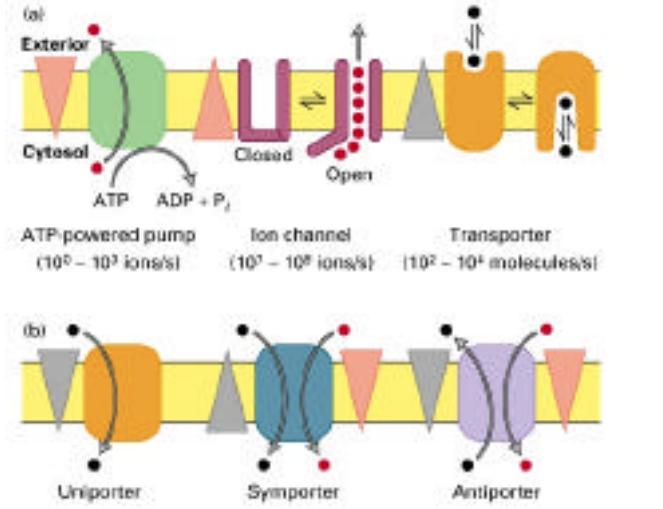

Fig. 3. Types of membrane transport

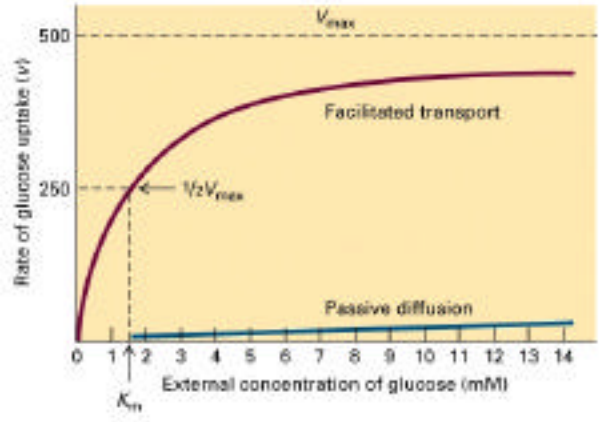

Fig. 4b. Facilitated diffusion versus passive diffusion.

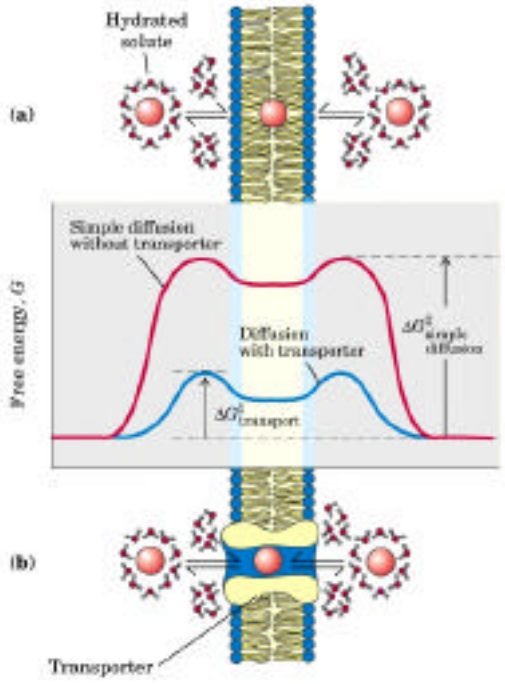

Fig. 4a. Facilitated diffusion versus passive diffusion.

2) Transport is specific. Each uniporter transports only a single species of molecule or a single group of closely related molecules

3) Transport occurs via a limited number of uniporter molecules, rather than throughout the surface of the phospholipid bilayer. Consequently there is a maximum transport rate V_{max} that is achieved when the concentration gradient across the membrane is very large and each uniporter is working in its maximal rate.

GLUT1, the glucose transporter of erythrocytes (Fig. 5). Transports Glucose into most mammalian cells. Alternates between two conformational states: in one, the glucose-binding site faces the outside of the membrane; in the other, a glucose-binding site faces the inside. Transports glucose 50,000 times faster than simple diffusion. It has a molecular weight of 45,000 Da with 12 putative transmembrane helical segments. Model predicts that the side-by-side assembly of several helices produces a transmembrane channel lined up with 12 hydrophilic residues that can hydrogen bond with glucose as it moves through the channel. The sequence of events that follow the initial binding step is shown in the figure 6c.

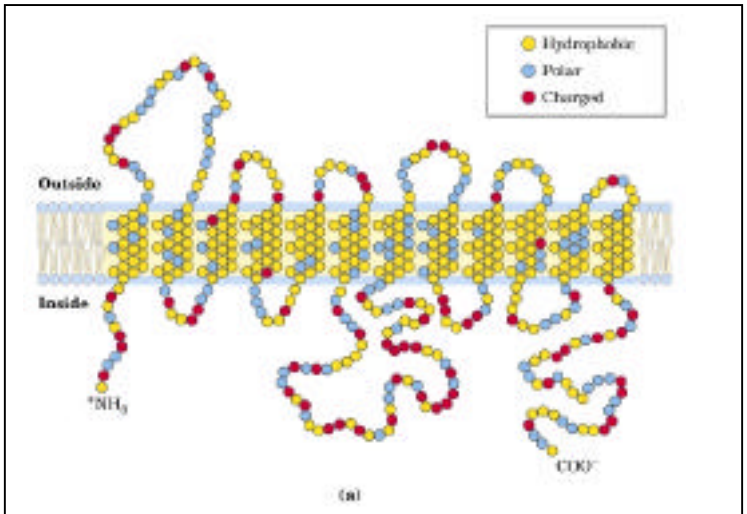

Fig. 5a. Secondary structure of GluT1

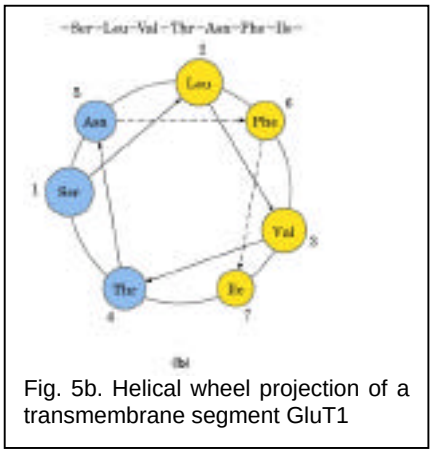

Fig. 5b. Helical wheel projection of a transmembrane segment GluT1

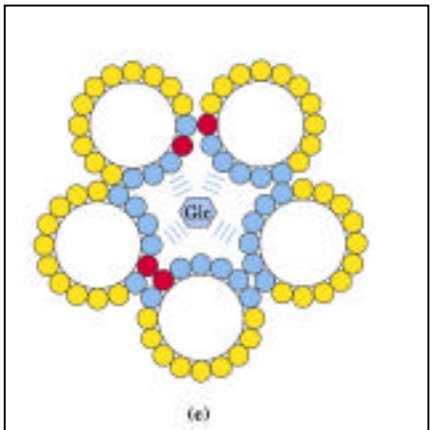

Fig. 5c. Association of apmhipathic helices forming a hydrophilic channel

Kinetics of GLUT1-catalyzed movement of glucose (Fig. 6.):
For simplicity let us assume that substance S is present initially only on one side of the membrane

$$S_{out} + \text{Transporter} \overset{K_m}{====} \text{S-Transporter Complex} \overset{V_{max}}{====} S_{in} + \text{Transporter}$$

Where K_m = Substrate-transporter binding constant
V_{max} = Maximum Transport rate of S into the cell

By similar derivation as in Michaelis-Menten equation, we can derive the following expression for v, the transport rate of S into the cell

$$v = V_{max} / 1 + K_m/C \qquad \text{(where } C = S_{out}\text{)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

V_{max} is the rate of transport if all the molecules of transporter contain a bound S, which occurs at high S_{out} concentrations

K_m is the substrate concentration at which half-maximal transport occurs across the membrane

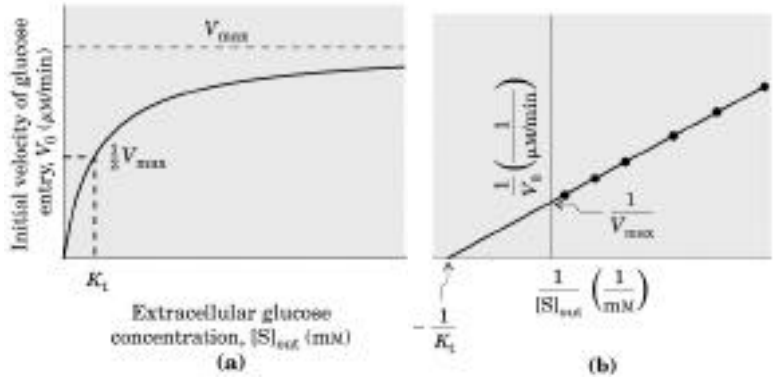

Fig. 6a,b. Kinetics of glucose transport in erythrocytes.

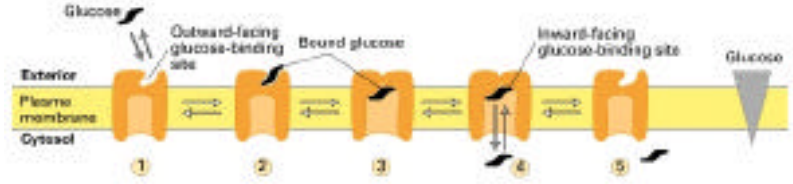

Fig. 6c. Uniport activity of GluT1

Channels

Transport water or specific types of ions down their concentration or electric potential gradients, an energetically favorable reaction. They form a protein-lined pathway across

the membrane and through which multiple water molecules or ions move simultaneously, in a single file at a very high rate-up to 10^8 per second.

General Properties of ion channels
Form hydrophilic pores
Always mediate downhill transport (passive)
Ion selective
Fluctuate between open and closed states (gated)
May be regulated

K^+ Channel (Fig. 7)

The structure of K^+ channel from the bacterium *Streptomyces lividans*, was determined by X-ray crystallography, in 1998. This bacterial ion channel is related in sequence to all K^+ channels and serves as the prototype for such channels, including the voltage-gated K^+ channel of the neurons. The similarity in sequence among the members of this family is highest in the pore region. The pore region contains the ion-selectivity filter that allows K^+ (radius 1.33Å) to pass 10,000 times faster than Na^+ (radius 0.95Å). The rate of K^+ passage is 10^8 ions per second.

Structure

Consists of four identical subunits that span the membrane and forms a cone surrounding the ion channel. The wide end of the cone faces the extracellular side. Each subunit has two transmembrane α-helices as well as a third shorter helix that contributes to the pore region. The outer cone is formed by one of the transmembrane α-helices of each subunit. The inner cone, formed by the other four transmembrane helices, surrounds the ion channel and cradles the ion selectivity filter. At the inner and outer plasma membrane surfaces, the entryways of the channel have several negatively charged amino acid residues, which presumably increase the local concentration of cations such as K^+ and Na^+. The ion path through the membrane begins (on the inner surface) as a wide, water-filled channel in which the ion can retain its hydration sphere. Further stabilization is provided by the short α−helices in the pore region of each subunit, with their carboxy-terminal end and the associated partial negative charges pointed at K^+ in the channel. About two third of the way through the membrane this channel narrows in the region of the selectivity filter, forcing the ion to give up its hydrating water molecules. Carbonyl oxygen atoms in the backbone of the selectivity filter replace the water molecule in the hydration sphere of K^+, forming a series of perfect coordination shells through which the K^+ moves. This favorable interaction is not possible with Na^+, which is too small to make contact with all the potential oxygen ligands. The preferential stabilization of K^+ is the basis for ion selectivity of the filter, and mutations that change residues in this part of the protein eliminate the channel's ion selectivity. K^+ ions pass through the filter in a single file. In the crystallographic structure two K^+ ions are visible, one at each end of the selectivity filter, about 7.5 Å apart. Other K^+ channels are similar in sequence, and presumably in structure and mechanism, to the S. lividans K^+ channel. The product of the Shaker gene, which is a K^+ channel in Drosophila, and K^+ channels in from nematode *Caenorhabditis elegans*, the ciliated protozoan Paramecium, and plant *Arabidopsis*

thaliana, all have sequences very similar to those in the pore region of the *S. lividans* K^+ channel. Furthermore, the amino acid sequences of Na^+ and Ca^{2+} channels suggest that they, too, share some structural and functional similarities with the bacterial K^+ channel. Determination of this channel's structure is therefore a landmark in channel biochemistry.

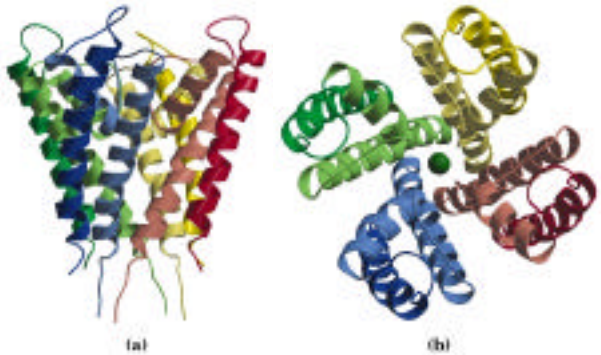

Fig. 7a & b. Structure of K^+ Channel of ***Streptomyces lividans*** (a) viewed in the plane of the membrane (c) viewed perpendicular to plane of the membrane

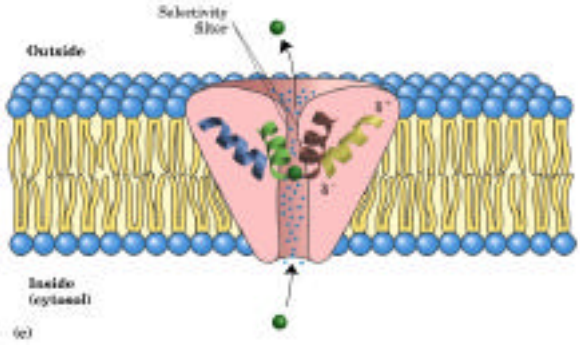

Fig. 7c. Structure of K^+ Channel of ***Streptomyces lividans***. Cross-section showing the water-filled channel

Gating of Channels

Channels are not continuously open. They have gates, which open briefly and close again, in response to specific stimulus. The main types of stimuli are

- Voltage across the membrane (voltage-gated)
- Mechanical stress (mechanically gated)
- Binding of ligand (ligand-gated)
  - Ligand could be extracellular (neurotransmitter) or intracellular (ATP)
  - Phosphorylation

Voltage-gated cation channels (Fig. 8)

Voltage-gated ion channels are responsible for generation of action potential in electrically excitable cells.

Voltage-gated Na^+ channels. Voltage-gated Na^+ channels in the plasma membrane of neurons and myocytes of heart and skeletal muscle sense electrical gradients across the membrane and respond by opening or closing. Very selective for Na^+ over other monovalent or divalent cations (by a factor of 100 or more) and have a very high flux rate ($> 10^7$ ions per second). Normally in the closed conformation Na^+ channels are activated (opened) by reduction in the transmembrane electrical potential. They then undergo very rapid inactivation. Within milliseconds of the opening, the channel closes and remains inactive for many milliseconds. Activation followed by inactivation of Na^+ channels is the basis for signaling by neurons. Single large polypeptide (1840 amino acid) organized into four domains clustered around a central channel, provide a path for Na^+ through the membrane. The path is made sodium specific by pore region composed of the segments between transmembrane helices5 and 6 of each domain, which fold into channel. This segment is believed to move into the membrane in response to changes in the transmembrane voltage, from the resting potential of about –60 mV (inside negative) to about +30 mV. The movement of helix 4 triggers opening of the channel. Inactivation of

the channel is believed to occur by a ball-and-chain mechanism. A protein domain on the cytosolic surface of the Na^+ channel, called the inactivation gate (the ball), is tethered to the channel by a short segment of polypeptide (the chain). This domain is free to move when the channel is closed, but when it opens, a site on the inner surface of the channel becomes available for the tethered ball to bind, blocking the channel. The length of the tether appears to determine how long an ion channel stays open; the longer the tether, the longer the open period. Inactivation of other ion channels may occur by a similar mechanism.

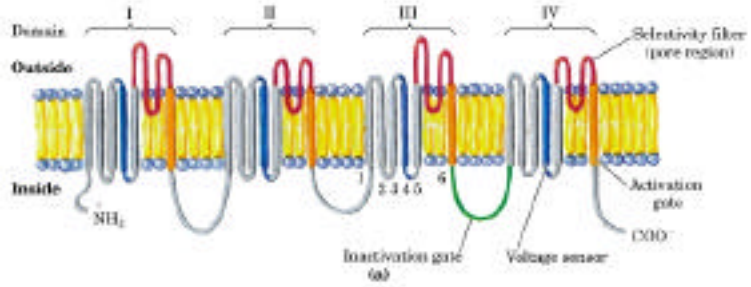

Fig. 8a. Voltage-gated Na^+ channel of neurons. Four homologous domains of α subunit.

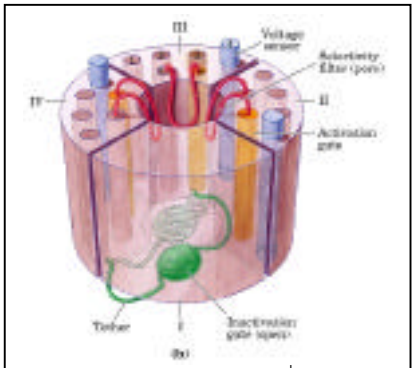

Fig. 8b. Voltage-gated Na^+ channel of neurons. Four domains are wrapped around a central hydrophobic channel.

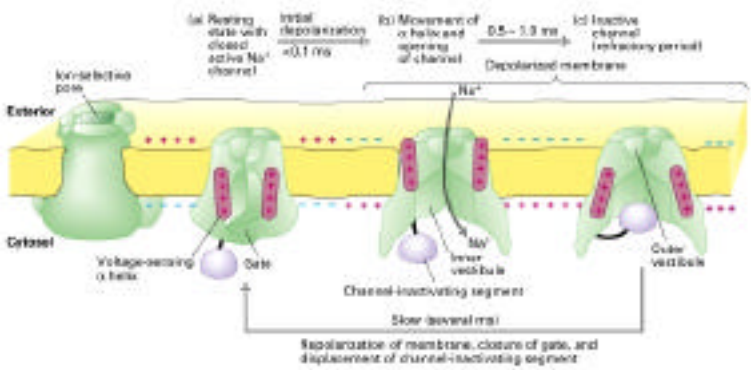

Fig. 8c. Voltage-gated Na^+ channel of neurons. Voltage-sensing mechanism

<u>Transmitter gated ion channels (Fig. 9)</u>

Converts chemical signal to electrical ones at the chemical synapses. Signals could be excitatory (acetylcholine, serotonin and glutamine) or inhibitory (γ-amino butyric acid and glycine).

Acetylcholine receptors at the neuromascular junction. Nicotinic Acetylcholine Receptor: Essential for the passage of an electrical signal from a motor neuron to a muscle fiber at the neuromascular junction. Acetylcholine released by the motor neuron diffuses a few micrometers to the plasma membrane of a myocyte, where it binds to the acetylcholine receptor. This forces a conformational change in the receptor, causing the ion channel intrinsic to the receptor to open. The resulting inward movement of positive charges depolarizes the plasma membrane of the myocyte, triggering contraction. The acetylcholine receptors allow Na^+, Ca^{2+} and K^+ to pass through with equal ease, but other

cations and all small anions are unable to pass. It has a gate that opens in response to stimulation by acetylcholine, and an intrinsic timing mechanism that closes the gate after a split second. These receptors are major targets for phsychoactive drugs.

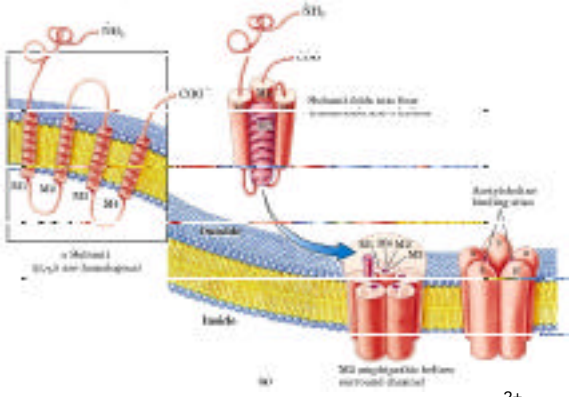

Fig. 9a. Acetylcholine receptor ion channel for Ca^{2+}, Na^{+} and K^{+}.

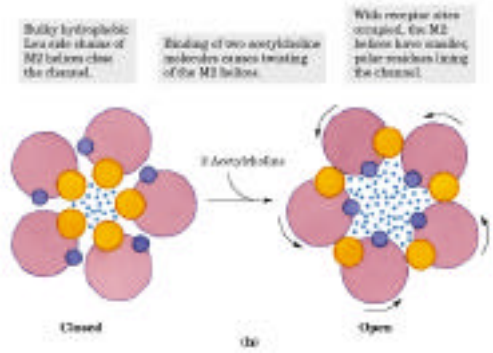

Fig. 9b. Acetylcholine receptor ion channel for Ca^{2+}, Na^{+} and K^{+}. Top view of a cross-section.

Aquaporin mediates passage of water

Provides channels for rapid movement of water molecules across plasma membrane. Erythrocytes, which swell and shrink rapidly in response to extracellular osmolarity, have a high density of aquaporin in their plasma membrane. Proximal renal tubule cells, which reabsorb water during urine formation, are rich in aquaporins. All aquaporins are membrane proteins with 6 transmembrane helical segments.

AQP-1 (Fig. 10). Four monomers of molecular weight 28,000 Da form a tetrameric transmembrane channel lined with hydrophilic side chains and having sufficient diameter (3 Å) to allow passage of water molecules in a single file. Water flows through at the rate of about 5 x 10^{8} molecules per second. The low activation energy for passage of water through aquaporin channels ($\Delta G^{\#}$ <15kJ/mol) suggests that water moves through the channel in a continuous stream. Direction of flow is directed by the osmotic gradient. Does not allow passage of ions or other small solutes.

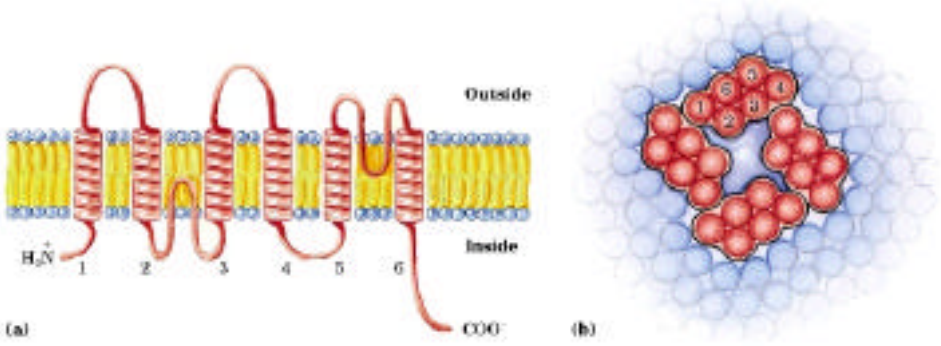

Fig. 10. Aquaporin (a) proposed transmembrane topology (b) proposed structure of the aquaporin channel.

table 12–3

Aquaporins

Aquaporin	Roles and location
AQP-1	Fluid reabsorption in proximal renal tubule; secretion of aqueous humor in eye and cerebrospinal fluid in central nervous system; water homeostasis in lung
AQP-2	Water permeability in renal collecting duct (mutations produce nephrogenic diabetes insipidus)
AQP-3	Water retention in renal collecting duct
AQP-4	Reabsorption of cerebrospinal fluid in central nervous system; regulation of brain edema
AQP-5	Fluid secretion in salivary glands, lachrymal glands, and alveolar epithelium of lung
γ-TIP	Water uptake by plant vacuole, regulating turgor pressure

Source: King, L.S. & Agre, P. (1996) Pathophysiology of the aquaporin water channels. *Annu. Rev. Physiol.* **58,** 619–648.

Porins are transmembrane channels for small molecules (Fig. 11)

In the outer membrane of gram-negative bacteria such as *E. coli*, protein channels called porins allow the passage of molecules much larger than ions, but by a mechanism more like a gated channel than a transporter. The porin FhuA serves in *E. coli* to bring iron (in the form of a chelate of ferrichrome) from the extracellular medium across the outer membrane and into the periplasmic space. FhuA is composed of a large 22-stranded b-barrel domain of about 560 residues and an amino-terminal cork domain of 160 residues, which normally obstructs the barrel and keeps the channel closed. Binding of the ferrichrome complex to a specific site on the outer side of the complex triggers allosteric changes that move the ferrichrome into the barrel and allow interaction with the FhuA protein with proteins of the inner membrane and periplasm. This interaction moves the cork out of the barrel and allows passage of the ferrichrome through the channel. With emptying of the ferrichrome binding site, the changes are reversed and the channel closes.

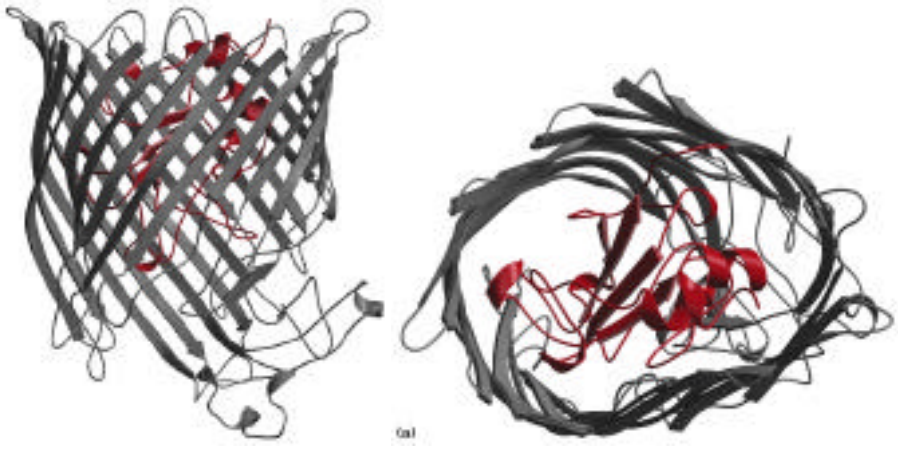

Fig. 11. Porin FhuA with transmembrane β-barrel structure; (a) side view; (b) top view.

Nuclear Pores (Fig. 12)

In all eukaryotic cells, the nuclear envelope is perforated by many pores, through which water-soluble molecules enter and leave the nucleus. Each pore is formed from an elaborate structure termed nuclear pore complex (NPC), which can selectively transport macromolecules across the nuclear envelope. Nuclear pore complexes actively transport macromolecules between the nucleus and the cytoplasm. They are immense in molecular standards (about 12 million daltons). It is made up of multiple copies of 50-100 different proteins. A central NPC nuclear ring supports eight = 100nm long filaments whose distal ends are joined by a terminal ring, forming a structure called nuclear basket. The nuclear ring is also attached to the nuclear lamina, a network of intermediate filaments that extends over the inner surface of the nuclear envelope. Ions, small metabolites, and globular proteins up to ≈ 60 kDa diffuse through water-filled channels in the nuclear pore complex. These pore behaves a if they are ≈ 9Å in diameter. However, large proteins and ribonucleoprotein complexes, up to ≈ 25nm in diameter cannot diffuse in and out of the nucleus; rather they are actively transported through the central plug of the nuclear pore complex. The nuclear pore acts as a gated channel through which these macromolecules are selectively transported in and out of the nucleus.

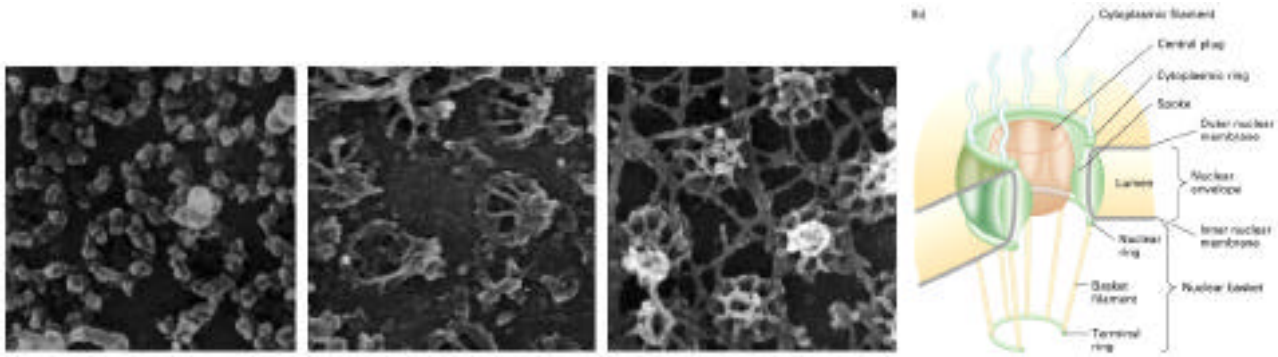

Fig. 12. Nuclear pore complex.

ACTIVE TRANSPORT

1) Primary pumps

Couples an energetically favorable reaction to transport against the gradient.

a) ATP coupled pumps (Fig. 13) Use energy derived from ATP hydrolysis to move ions or small molecules across the membrane against a chemical concentration gradient or electric potential. Couples energetically favorable reaction of ATP hydrolysis to energetically unfavorable movement of solutes against the concentration gradient. There are four major types of ATP-coupled pumps.

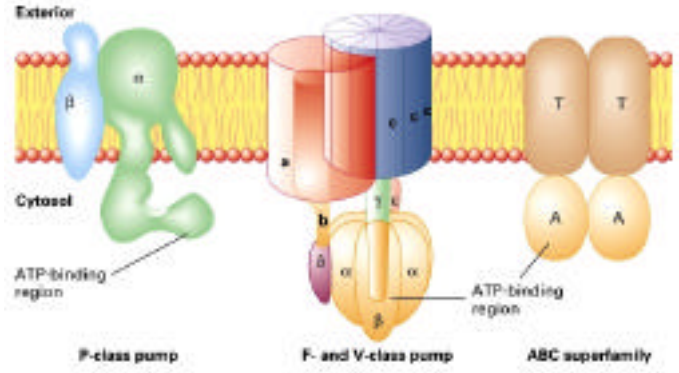

Fig. 13. Four classes of ATP powered transport proteins.

i) P-type ATPase:

Contains a transmembrane catalytic α-subunit, which contains an ATP-binding site and usually a smaller β subunit, which may have a regulatory functions. During transport at least one of the α subunit is phosphorylated (hence labeled P), and the transported ions are thought to move through the phosphorylated subunit

Examples:

Na^+/K^+ ATPase of the animal cells plasma membrane. Maintains the Na^+ and K^+ gradients typical of animal cells (Fig. 14.)

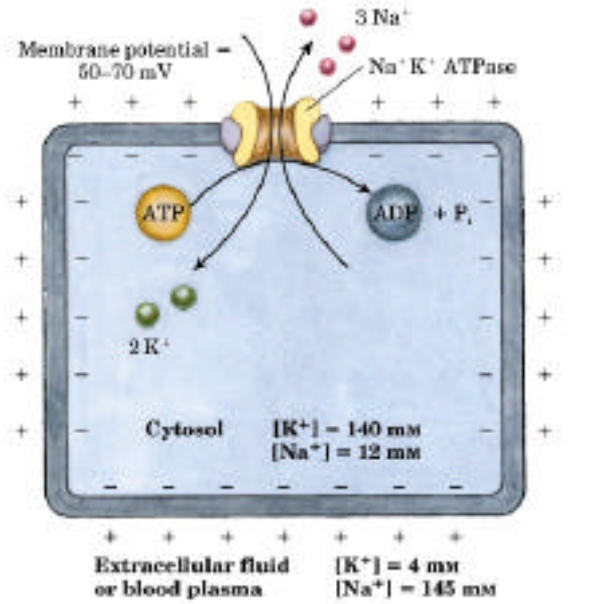

Fig. 14a. Na^+/K^+ ATPase.

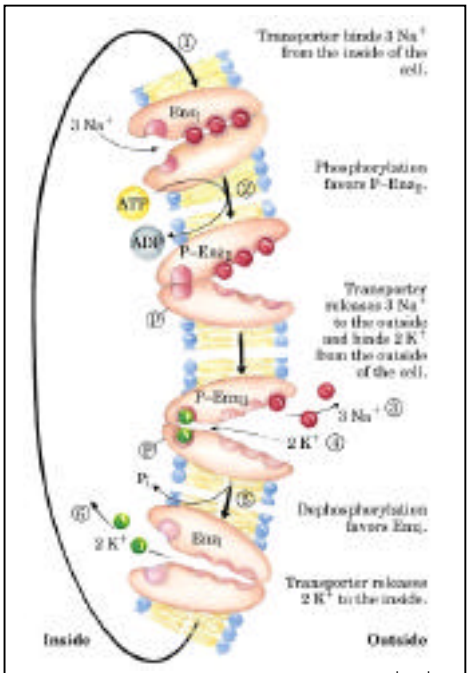

Fig. 14b. Mechanism of action of Na^+/K^+ ATPase.

Ca^{2+} ATPases, that pump Ca^{2+} ions out of the cytosol into the external medium or into the lumen of sarcoplasmic reticulum. The cytosolic concentration of free Ca^{2+} is generally about 100 nM, far below that in the surrounding medium (Fig. 15).

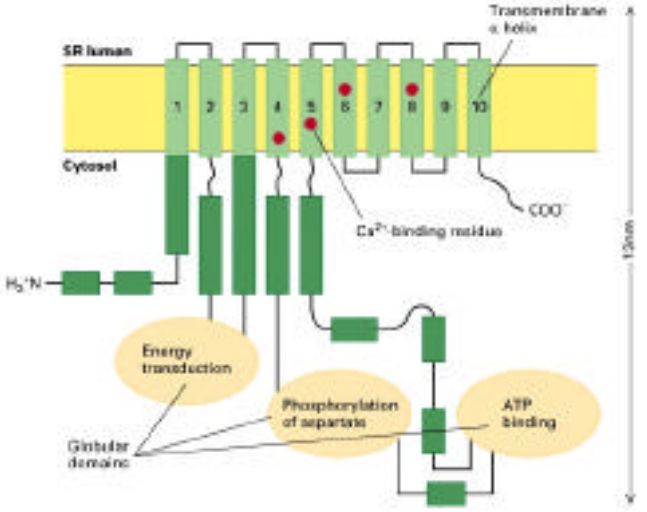

Fig. 15a. Catalytic (α) subunit of muscle Ca^{+} ATPase

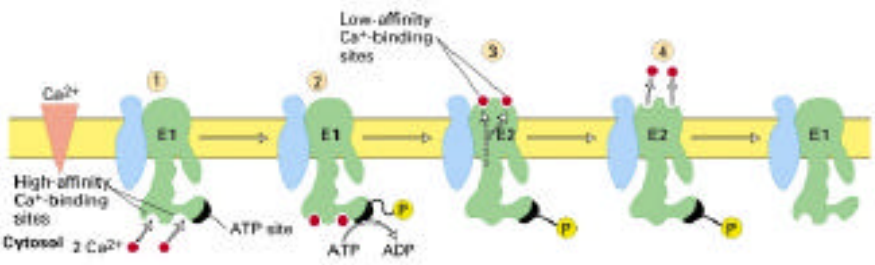

Fig. 15b. Mechanism of action of the muscle Ca^{+} ATPase.

K^{+}/H^{+} ATPases of the acid secreting cells of the mammalian stomach. Pumps K^{+} in and H^{+} out of the cells.

H+ pumps that maintains the membrane electric potential in plant, fungal, and bacterial cells.

ii) F-type and V-type ATPases

F-type and V-type ion pumps are similar to each other but unrelated to and more complicated than the P-type pumps. Contains at least three kinds of transmembrane proteins and five kinds of extrinsic polypeptides that form the cytosolic domain. Several of the transmembrane and extrinsic subunits in F-type and V-type pumps exhibit sequence homology, and each pair of homologous subunits is thought to have evolved from a common polypeptide (Fig. 16).

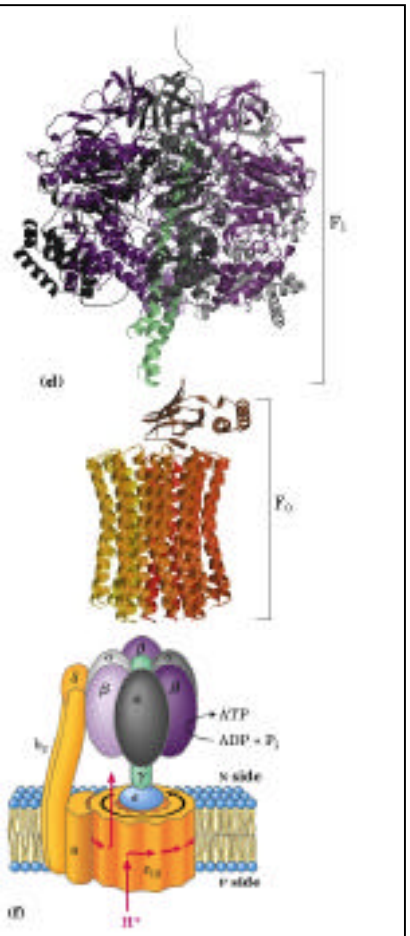

Fig. 17.1, Structure of mitochondrial F_0F_1 ATP synthase complex.

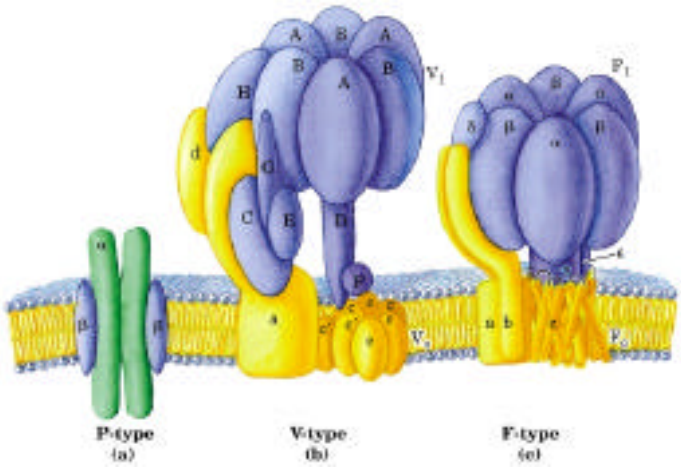

Fig. 16. Subunit structures of three ion-translocating ATPases

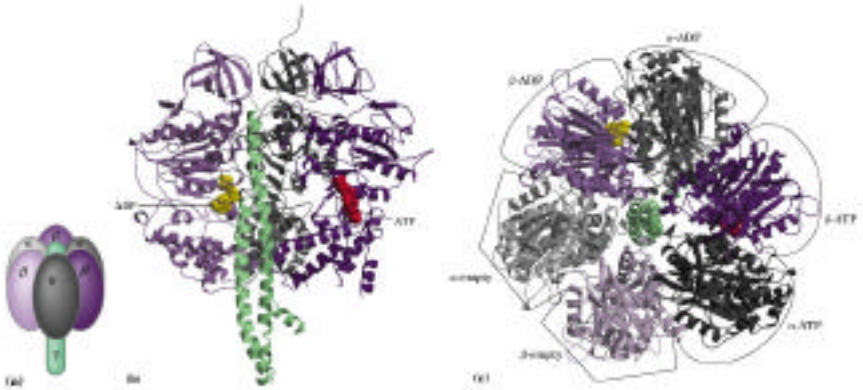

Fig. 17.2, Structure of mitochondrial F_1 complex.

V-type ATPases pump proton in a process that does not involve phosphoprotein intermediate. V-type generally function to maintain the low pH of plant vacuoles and the lysosomes and other acidic vesicles of the animal cells. Uses energy released by ATP hydrolysis to pump protons from the cytosolic to the exoplasmic face of the membrane, against the proton electrochemical gradient.

F-type pumps are found in bacterial plasma membranes and in mitochondria and chloroplasts. They generally function to power the synthesis of ATP from ADP and Pi by movement of protons from the endoplasmic to the cytosolic face of the membrane down the proton gradient.

ATP Synthase comprises a proton channel (F_0) and ATPase (F_1). Both are oligomeric proteins. F_0 is located within the membrane and contains a transmembrane channel through protons flow toward F_1, most of which extends into mitochondrial matrix. The F_0 components contain three types of subunits, a, b, and c; in bacteria, the subunit composition is $a_1b_2c_{9\text{-}12}$. When F_0 is experimentally incorporated into liposomes, the

permeability of the vesicles to H^+ is greatly stimulated, indicating that it indeed forms a proton channel. The F_1 portion is a water-soluble complex of five distinct peptides with composition of $\alpha_3\beta_3\gamma\delta\varepsilon$. The α and β subunits associate in alternating order to form a hexamer, αβαβαβ or $(\alpha\beta)_3$ This hexamer rests atop a single long γ subunit, whose lower part is a coiled coil that fits into the c-subunit ring of F_0. The ε subunit is attached to the γ and probably also contacts the c subunits of the F_0. The δ subunit of the F_1 complex contacts the b subunit of the F_0 complex; together these subunits form a rigid "stator" that prevents the $(\alpha\beta)_3$ hexamer from rotating while it rests on the γ subunit (Fig. 17 & 18a).

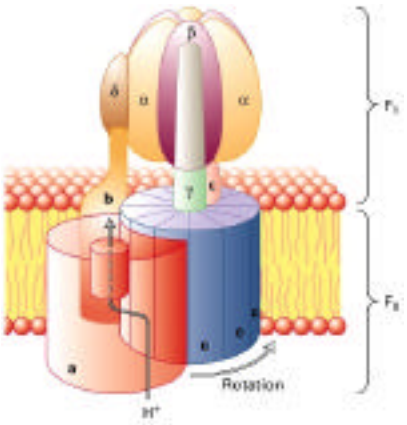

Fig. 18a. ATP Synthase, The F0F1 complex.

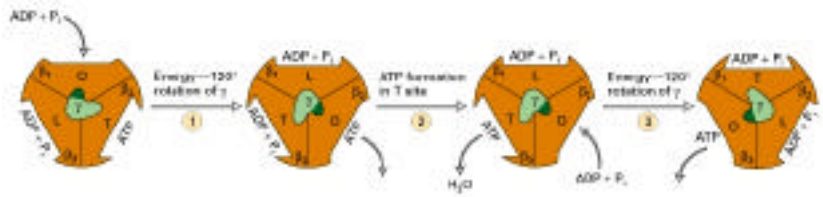

Fig. 18b. ATP Synthase, The F0F1 complex. The binding change mechanism of ATP synthesis from ADP and Pi (phosphate).

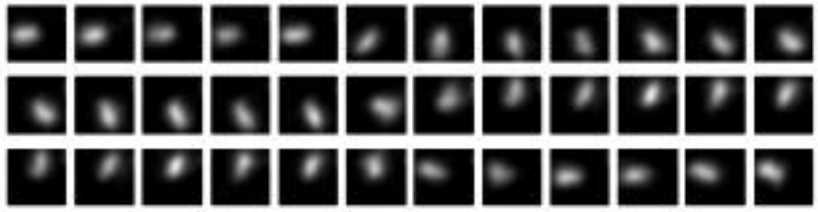

Fig. 18d. Rotation of F_0 and γ subunit experimentally demonstrated

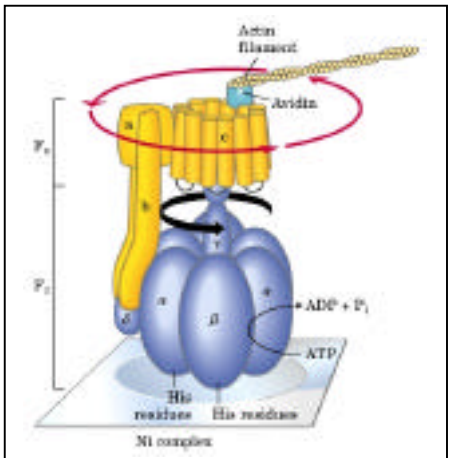

Fig. 18c. Rotation of F_0 and γ subunit experimentally demonstrated.

Reversibility of F-type ATPases.
They can catalyze uphill transmembrane passage of protons driven by ATP hydrolysis, as well as the reverse reaction, in which downhill proton flow drives ATP synthesis.

iii) ABC-type

Larger and more diverse than the other types of transport ATPases. Referred to as the ATP-binding cassette (ABC) superfamily, this class includes more than 100 different transport proteins found in organisms ranging from bacteria to humans. Each ABC protein is specific for a single substrate or a group of related substrates including ions, sugar molecules, polypeptides, polysaccharides, and even proteins. All ABC proteins share a common structural organization consisting of four "core" domains: Two transmembrane (T) domains, forming the passageway through which transported

molecules cross the membrane, and two cytosolic ATP-binding (A) domains. In some ABC proteins the core domains are present in four different polypeptides; in others, the core domains are fused into one or two multi-domain polypeptides.

Examples:
Bacterial plasma membrane **permeases** (Fig. 19).
Mammalian MDR transport proteins (Fig. 20).
Cystic Fibrosis Conductance regulator protein

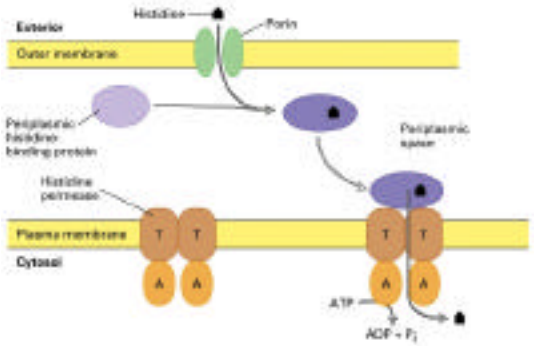

Fig. 19. Bacterial ABC proteins (permeases)

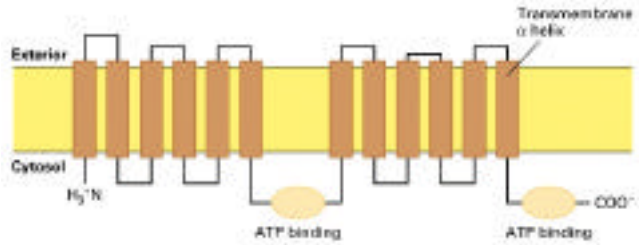

Fig. 20. Structural model for mammalian MDR1 protein

All classes of ATP-powered pumps have one or more binding sites for ATP, and these are always on the cytosolic side face of the membrane. Normally do not hydrolyze ATP unless transport substrates are in contact with the protein. They collect the free energy released during ATP hydrolysis and use it to move ions or other molecules uphill against a potential or concentration gradient.

b) Light Driven

Bacteriorhodopsin. Bacteriorhodopsin is a light-driven proton pump found in the purple membranes of *Halobacterium halobium*. Bacteriorhodopsin is one of the best-studied membrane-transport proteins. It has seven transmembrane α-helices which are connected by non-helical loops at the inner and outer face of the membrane. The transmembrane regions are rich in hydrophobic amino acid residues. Hydrophobic interactions between the non-polar amino acid residues and the fatty acyl groups of the membrane lipids firmly anchor the protein in the membrane. The seven helices are clustered together to form the transmembrane pathway for proton movement. At the reaction center, bacteriorhodopsin contains retinal (the aldehyde derivative of vitamin A) as a prosthetic group. When the cells are illuminated, all-trans-retinal bound to bacteriorhodopsin absorbs a photon and undergoes photoisomerization to 13-cis-retinal. The restoration of the all-trans-retinal is accompanied by an outward movement of H^+ through the plasma membrane. It is the simplest light-driven proton pump. The difference in the three-dimensional structure of the of bacteriorhodopsin in the dark and after illumination suggests a pathway by which a concerted series of protons "hops" could effectively move a proton across the membrane as the conformational changes occur. The chromophore retinal is bound through a

Schiff-base linkage to the ε-amino group of a Lysine residue. In the dark, N of this Schiff base is protonated, but photoisomerization of retinal lowers the pKa of this group and releases its proton to a nearby Asp residue, triggering a series of proton hops that ultimately results in the release of proton at the outer surface of the membrane. The resulting electrochemical potential drives protons back into the cell through a membrane ATP synthase complex. Therefore, halobacteria can use light to supplement the ATP synthesized by oxidative phosphorylation when O_2 is limited (Fig. 22).

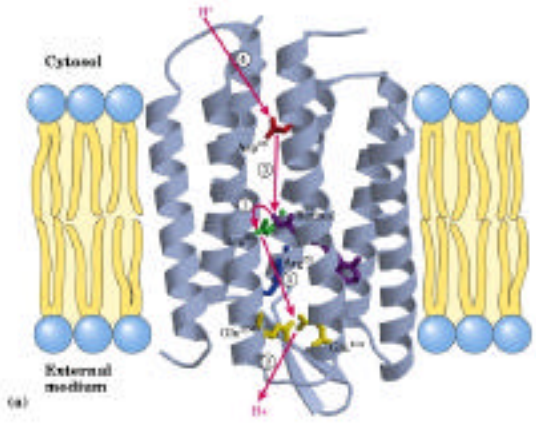

Fig. 22a. Light-driven proton pumping by bacteriorhodopsin

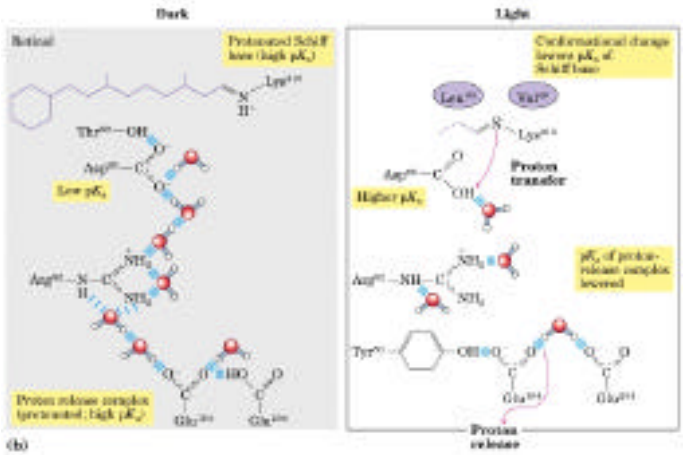

Fig. 22b. Light-driven proton pumping by bacteriorhodopsin.

c) Electron flow-driven

Proteins that catalyze transport of solute (eg. ion) energized by the flow of electrons from a reduced substrate to an oxidized substrate. The discovery in 1948 by Eugene Kennedy and Albert Lehninger that mitochondria are the sites of oxidative phosphorylation in eukaryotes marked the beginning of modern phase of studies in biological energy transductions. Mitochondria, like bacteria, have two membranes. The outer mitochondrial membrane is permeable to small molecules and ions, which move freely through transmembrane channels formed by a family of integral membrane proteins called porins. The inner membrane is impermeable to most small molecules and ions, including protons (H^+): the only species that cross the inner membrane are those for which there are specific transporters. The inner membrane bears the components of the respiratory chain and the ATP synthase. The inner membrane of mitochondria is the site for oxidative phosphorylation. The electron carriers of the respiratory chain are organized into membrane-embedded supramolecular complexes. There are four unique electron carrier complexes, each capable of catalyzing electron transfer through a portion of the chain. Complexes I and II catalyze electron transfer to ubiquinone from two different electron donors: NADH (Complex I) and succinate (Complex II). Complex III carries electrons from ubiquinone to cytochrome c, and Complex IV completes the sequence by transferring electrons from cytochrome c to O_2. The transfer of two electrons from NADH through the respiratory chain to molecular oxygen has a negative standard free-energy change, much of this energy is utilized to pump protons out of the

matrix. For each pair of electrons transferred to O_2, four protons are pumped out by Complex I, four by Complex III, and two by Complex IV (Fig. 23).

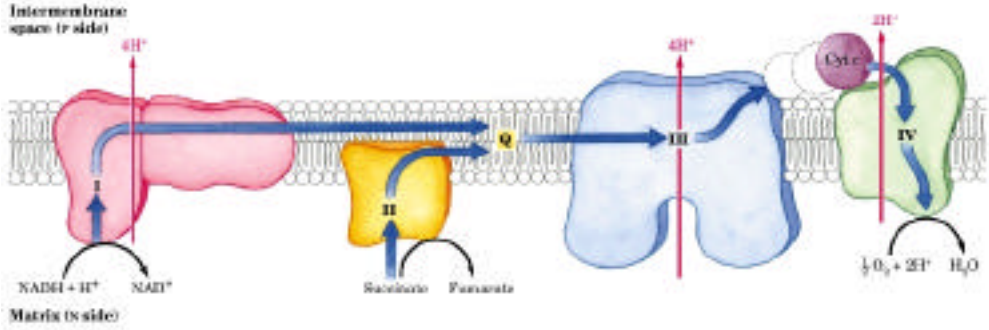

Fig. 23. Flow of electrons and proton pumping through the Electron Transfer Chain.

Proton-motive force and Membrane potential:

The electrochemical energy inherent in this difference in proton concentration and separation of charge represents a temporary conservation of much of the energy of electron transfer. This energy stored in such a gradient is termed the proton-motive force. Proton-motive force has two components: 1) the chemical potential energy due to the difference in concentration of a chemical species (H^+) between the two regions separated by the membrane. (2) the electrical potential energy that results from the separation of charge when proton moves across the membrane without a counter ion. When protons flow back spontaneously down the electrochemical gradient, energy is made available to do work. In Mitochondria, chloroplast and aerobic bacteria, the electrochemical energy in the proton gradient drives the synthesis of ATP from ADP and Pi in ATP synthase. The chemiosmotic model proposed by Peter Mitchell is the paradigm for this mechanism. According to the model, the electrochemical energy inherent in the difference in proton concentration and separation of charge across the mitochondrial inner membrane is the driving force for synthesis of ATP. The proton-motive force drives the synthesis of ATP as protons flow passively back into the matrix through a proton pore associated with ATP

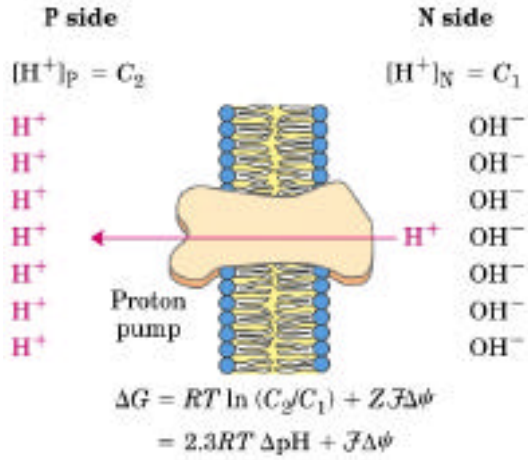

Fig. 24. Proton-motive force

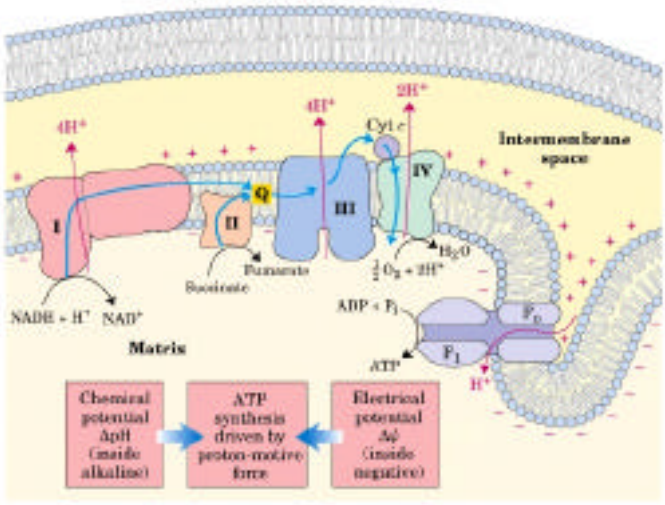

Fig. 25a. Chemiosmotic model proposed by Peter Mitchell

synthase (Fig. 24 & Fig. 25).

2) <u>Secondary active transporters or cotransporters</u>

These proteins use energy stored in the electrochemical gradient of Na^+ or H^+ ions to power uphill movement of another substance, a process called cotransport. When the transported molecule and the cotransported ion move in the same direction, the process is called symport; when they move in opposite directions, the process is called antiport. Membrane potential in animal cells is the driving force for secondary transporters, where as the proton-motive force play an important role in bacterial cotransporters. Secondary carriers couple the movement of one type of ion or molecules against its concentration gradient (uphill) to the movement of a different ion or molecule down its concentration gradient (downhill). These proteins are also called cotransporters (Fig. 26).

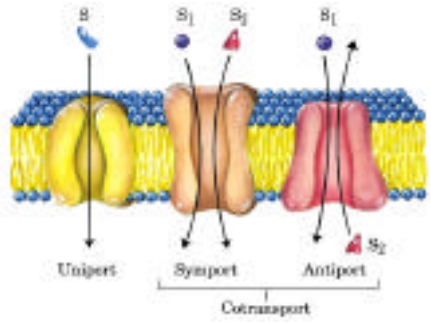

Fig. 26. Secondary transporter or cotransporters compared with uniporter

Table 12–5

Cotransport Systems Driven by Gradients of Na^+ or H^+

Organism or tissue	Transported solute (moving against its gradient)	Cotransported solute (moving down its gradient)	Type of transport
E. coli	Lactose	H^+	Symport
	Proline	H^+	Symport
	Dicarboxylic acids	H^+	Symport
Intestine, kidney of vertebrates	Glucose	Na^+	Symport
	Amino acids	Na^+	Symport
Vertebrate cells (many types)	Ca^{2+}	Na^+	Antiport
Higher plants	K^+	H^+	Antiport
Fungi (*Neurospora*)	K^+	H^+	Antiport

Table 3. Cotransporters driven by gradients of H^+ and Na^+..

Types of Secondary transporters

a) Symporter

Na^+/HCO_3^- symporter (decreases pH of the cytosol by extruding HCO_3^-)
Na^+-linked symporters import amino acids and glucose into many animal cells (Fig. 27).

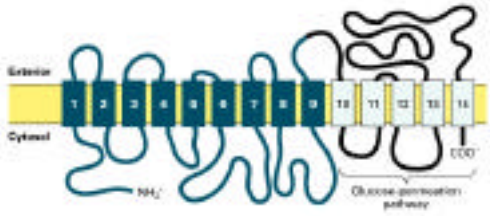

Fig. 27a. Secondary structure of Na^+/Glucose symporter

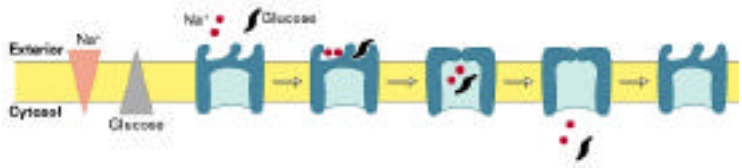

Fig. 27b. Mechanism of action for Na^+/Glucose symporter

H^+-linked lactose symporter in *E. coli* (Fig. 28).

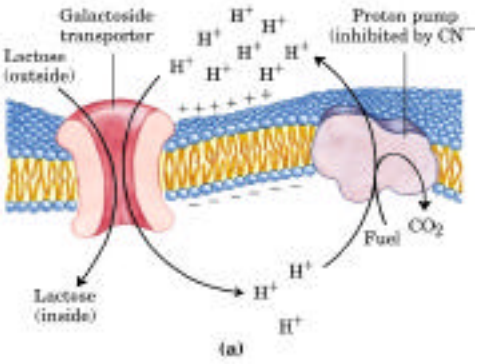

Fig. 28a. H+-coupled lactose uptake in E. coli

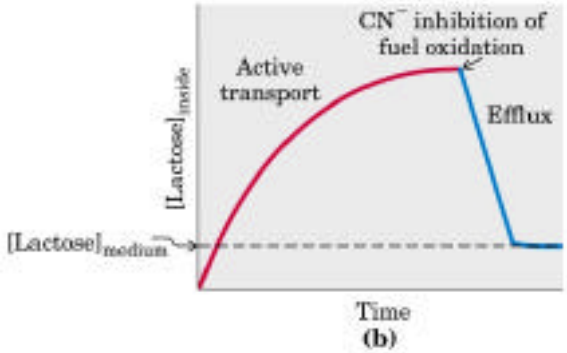

Fig. 28b. H^+-coupled lactose uptake in E. col1. Effect of Cyanide

b) Antiporter

Na^+/Ca^{2+} antiporter of cardiac muscle cells
Na^+-H+-exchanger (Couples influx of Na+ to efflux of H+)
Cl^-/HCO_3^- Exchanger of erythrocytes (Fig. 29)

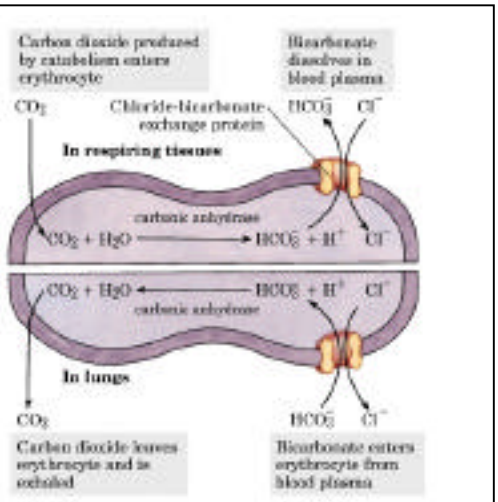

Fig. 29. Na^+ -driven Cl^-/HCO_3^- exchanger of the erythrocyte membrane

Na^+/Ca^{2+} antiporter exports Ca^{2+} from cardiac muscle cells. In cardiac muscle plays crucial role in maintaining a low concentration of Ca^{2+} in the cytosol.

Ionophores and membrane permeability

Ionophores are small hydrophobic molecules that dissolve in lipid bilayers and increase their permeability to specific inorganic ions. Most ionophores are synthesized by microorganisms. They are widely used by cell biologists as tools to increase the ion permeability of membranes.

Mobile ion carriers:

Valinomycin

It is a mobile ion carrier. Ring-shaped polymer that reversibly binds and transports K^+ down its electrochemical gradient. It picks up K^+ on one side of the membrane, diffuse across the bilayer, and release K^+ on the other side (Fig. 30).

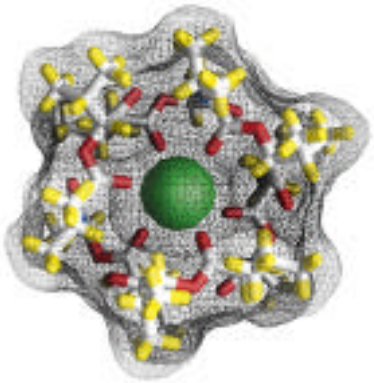

Fig. 30. Valinomycin, a peptide ionophore that binds K^+ ion.

Channel formers:

Gramicidin

Channel forming ionophore. Linear peptide of 15 amino acid residues, all with hydrophobic side chains. Two gramicidin molecules come together end to end across the bilayer to form a transmembrane channel. Selectively allows monovalent cations to flow down their electrochemical gradients.

Origin of Membrane potential in animal cell:

Similar to generation of proton-motive force, a membrane potential arises when there is a difference in electrical charge on the two sides of a membrane, due to slight excess of positive ions over negative on one side and a slight deficit on the other. Such charge differences can result from active electrogenic pumping and from passive ion diffusion.

Mediated mainly by

- Plasma membrane Na^+/K^+-ATPase
- Plasma membrane K^+ leak channels
- Role of Cl^- channel is negligible

Na^+/K^+-ATPase helps maintain osmotic balance across animal cell membrane by keeping the intracellular concentration of Na^+ low. The K^+ ion that is pumped into the cell by the Na^+/K^+-ATPase balance the charged carried by negatively charged organic molecules that are plentiful inside the cell. K^+ can also freely move in and out of the cell through K^+ leak channels in the plasma membrane. Since the concentration of K^+ is high inside the cell and low outside, K^+ will tend to leave the cell through the K^+ leak channel, driven by its concentration gradient. As K^+ moves out, it will leave behind unbalanced negative charge, thereby creating an electrical field, or membrane potential, which will oppose the further efflux of K^+. The net flux will come into a halt when membrane potential reaches a value where this electrical driving force of K^+ exactly balances the effect of its concentration gradient-that is, when the electrochemical gradient for K^+ is zero. Although Cl^- ions also equilibrate across the membrane, because their charge is negative, the membrane potential keeps most of these ions out of the cell. The equilibrium condition, in which there is no net flow of ions across the plasma membrane, defines the resting potential (Fig. 31).

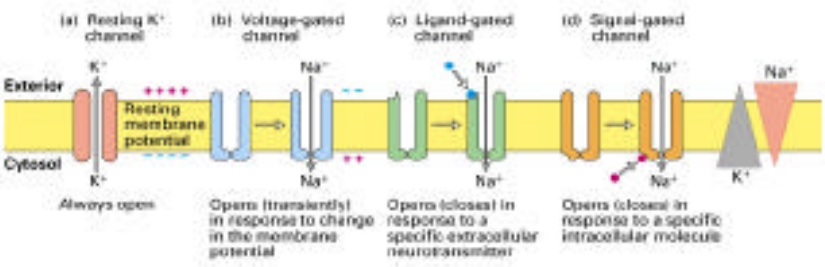

Fig. 31. Ion channels and membrane potential.

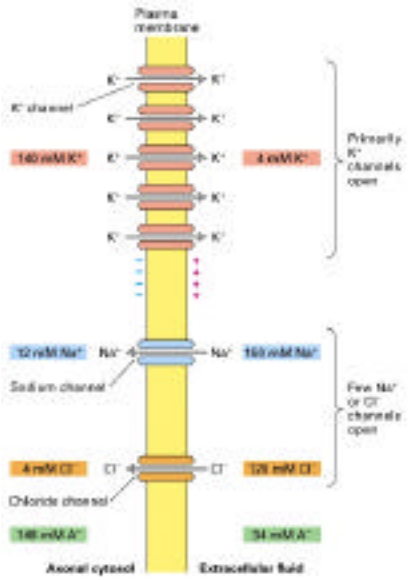

Fig. 32. Origin of the resting potential

Resting membrane potential (Fig. 32) (When the electrochemical potential for K^+ is 0) Nernst equation expresses the equilibrium condition quantitatively and makes it possible to calculate the theoretical resting membrane potential if the ratio of internal and external ion concentration is known.

$$V = RT/zF \ln C_o/C_i$$
$$= 2.3\ RT/zF \log_{10} C_o/C_i$$

V= the equilibrium potential in volts
C_o and Ci = outside and inside concentrations of the ion, respectively
R= the gas constant (2 cal mol^{-1} $^oK^{-1}$)
T = the absolute temperature (^oK)

F = Faraday's constant (2.3 x 104 cal V^{-1})
z = the valance (charge) of the ion
ln = logarithm to the base e

Overview of types of transport processes

Summary of transport types (Fig. 33).

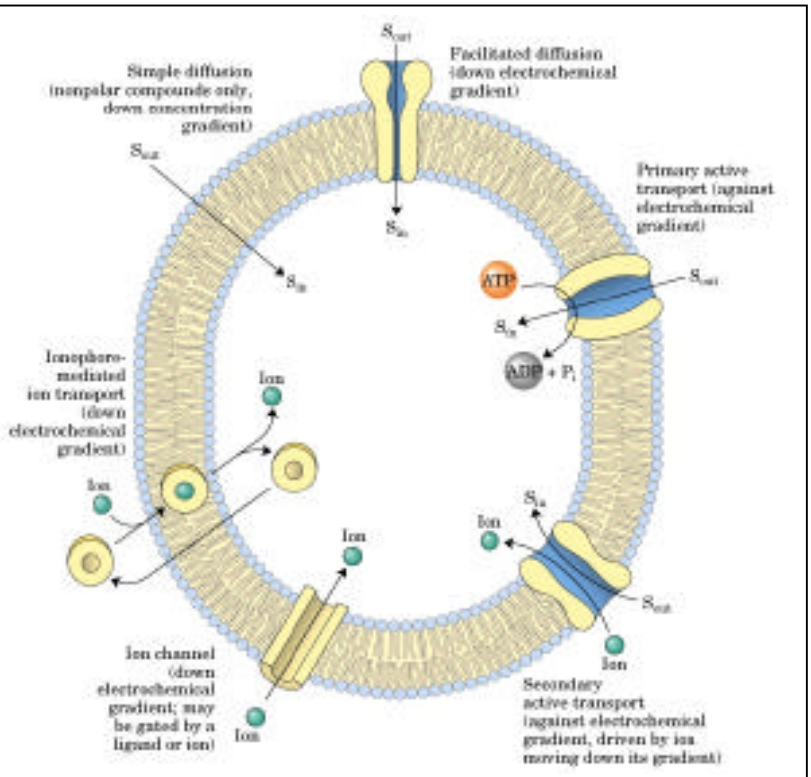

Fig. 33. Summary of transport types.

Regulation of Transport:

Transport can be regulated by
- Changes in transport driving force
- Modifications of endogenous transporters
- Increase in number of transporter molecules

Changes in Driving Force: Role of plasma membrane potential

In electrogenic transport, the steady-state accumulation of that substrate is influenced by the magnitude of the cell membrane potential. The kinetic parameters such as the K_m of the substrate or V_{max} of the transport may also be affected by membrane potential. Can be experimentally determined in membrane vesicles where the membrane potential can be varied by addition of membrane-permeable anions such as SCN^- or by addition of

valinomycin in experiments where the intravesicular and extravesicular concentration of K^+ are equal.

The short-term regulation of liver metabolism by glucagon represents one important example of a situation where membrane potential related alterations in transport kinetics are of physiological importance. Liver cell membrane has a low permeability to K^+ ions, and the consensus membrane potential of –35 mV. This is far from the K^+ diffusion potential of –70 mV and is mainly set up by Na^+/K^+ ATPase. One major effect of glucagon is to cause a rapid hyperpolarization of the liver cell membrane to about –50 mV. This may be due in part to opening of specific K^+ channels and in part to activation of the Na^+/K^+ exchanger by cAMP, presumably via phosphorylation event with a consequent increase in the intracellular Na^+ concentration and stimulation of the electrogenic Na^+/K^+ ATPase. This hyperpolarization stimulates the rate of transport into liver cell of a number of important substrates. The initial phase is independent of protein synthesis and follows by a slow protein synthesis dependent induction of transport.

Alanine is a major substrate for gluconeogenesis in liver, and alanine transport is a major control step for gluconeogenesis. Epidermal growth factor stimulates both alanine transport and gluconeogenesis in hepatocytes. A single rapid phase of stimulation is observed, which is independent of protein synthesis. The stimulation is due to hyperpolarization of the membrane, via Epidermal Growth factor (EGF) stimulation of the Na^+/H^+ exchanger and subsequent activation of Na^+/K^+ ATPase.

Modifications of the transporter molecules

Covalent modifications of channels and carriers

Almost all membrane proteins are modified after synthesis. Changes may involve removal of leader sequence, glycosylation, and acylation. These are irreversible steps of the processing mechanism that ensure correct folding and sorting of the protein. In some cases chemical modifications modulate protein function by short-term regulatory mechanism. Most common is protein phosphorylation by kinases; formation of an ester bond between a phosphate group, donated by ATP, and the hydroxyl group of serine, threonine, or tyrosine side chains. This modification is reversible by the action of a number of phosphatases. Therefore, many regulatory pathways involve the activation of kinases and phosphatases.

Example:

Cystic Fibrosis Conductance Regulator (Fig. 33.1): CFTR is an ABC transporter. The proposed structure comprises two membrane spanning domains (each formed by 6 transmembrane segments) and a highly complex cytoplasmic region. The cytoplasmic domain is formed by a regulatory R-domain and by two highly homologous nucleotide-binding domains (NBD1 and NBD2). CFTR is an outwardly directed Cl^- channel. CAMP-dependent PKA (Phosphokinase A) phosphorylates R-domain of CFTR, and subsequent ATP binding/hydrolysis open the channel activity. PKA mediated

phosphorylation in certain serine residues of the R-domain stimulates the Cl^- fluxes. Addition of negative charge though phosphorylation is thought to an important mechanism in opening of the channel by changing electrostatic interactions among the various domains of the protein. When more than 5 serine residues were changed to aspartate (or glutamate) the CFTR channel opened in absence of cAMP.

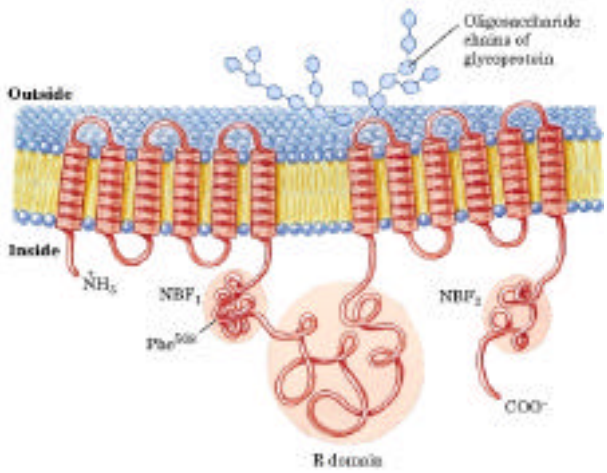

Fig. 33.1. Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

Oligomerization

Many transporters and channels are composed of distinct subunits. The different levels of oligomeric organization can produce profound changes in the transport process. The N^+/H^+ exchangers (NHE) are single-chain polypeptides, but are believed to work as oligomers. Change in intracellular pH may modulate the interaction between distinct molecules of NHE through H^+-modifier site in the N-terminal domain of the protein.

Allosteric interactions

In some cases allosteric interaction is of pivotal importance for the function of the transporter. Na^+/H^+ exchanger (NHE) has a cytoplasmic modifier region that is the site for allosteric interaction with protons. Under normal conditions (low cytoplasmic Ca^{2+}), the calmodulin binding site interacts with the modifier site, lowering the affinity of the carrier for cytoplasmic H^+. Upon cell stimulation, the increase in concentration of Ca^{2+} activates kinases, thus leading to calmodulin phosphorylation and binding, with subsequent removal of modifier site inhibition, decrease in transporter K_m for H^+, and exchange stimulation.

<u>Changing the repertoire of transport proteins</u>

In mammalian proteins the membrane content of transport proteins is not constant under all conditions. Transport activity can be stimulated by mechanisms, which involves de novo protein synthesis and the putative insertion of additional transport proteins into the cell membrane.

Hormonal regulation
The ubiquitous transporter System A is a Na^+ dependent, highly regulated mechanism for the uptake of small aliphatic amino acids in mammalian cells. System A activity is induced by a number of hormones, in particular glucagon, catecholamines, and glucocorticoids. Stimulation by glucagon has been most studied. Induction of System A by glucagon is protein synthesis–dependent and is sensitive to cyclohexamide (inhibitor of protein synthesis) and tunicamycin (inhibitor of glycosylation). The decay of glucagon induced activity of system A after the withdrawal of glucagon involves an additional newly synthesized protein necessary for degradation of the induced activity.

Induction by long-term substrate deprivation
A variety of mammalian cell types induce transport activity in response to substrate deprivation. In general, transport activity increases following substrate withdrawal after a lag period of some hours. Induction of transport activity depends on RNA and protein synthesis and is reversed over a certain period of time by readdition of substrate. A physiologically relevant example of adaptive regulation is that of phosphate transport in renal rush border membranes. Feeding animals a diet low in phosphate leads to an increase in renal phosphate reabsorption, and this can be attributed to an increase in the activity of renal Na^+-phosphate cotransport. Two Na^+-phosphate cotransporters have been cloned, Type I and Type II, of which the Type II shows properties consistent with phosphate transport in renal membranes.

Induction by exposure to hyperosmotic medium
Cells in kidney medulla are exposed to hypertonic conditions during the production of concentrated urine. Such cell respond by increasing the number of N^+- linked uptake of compounds such as Myo-inositol, betaine, and turine. These compounds are known as compatible osmolytes since they are relatively inert, do not interfere with cell function, and may exert a protective effect on protein conformation. The accumulation of these solutes increases the osmotic pressure and the consequent influx of water helps to restore the cell volume. A number of transport proteins catalyzing the Na^+ dependent accumulation of osmolytes have been cloned.

<u>Derangements in transport regulation</u>

Diabetes mellitus:

Type I. Glucose uptake in myocytes and adipocytes is mediated by the glucose transporter GluT4. Between meals the plasma membrane of these cells contain some GluT4, but most is sequestered in the membranes of small intracellular vesicles. Insulin released from the pancreas in response to high blood glucose triggers the movement of the intracellular vesicles to the plasma membrane, where they fuse, thus exposing GluT4 molecules on the outer surface of the cell. With more GluT4 molecules in action, the rate of glucose uptake increases 15-fold or more. When blood glucose level returns to normal, insulin release slows and most GluT4 molecules are removed from the plasma membrane and stored in vesicles (Fig. 34).

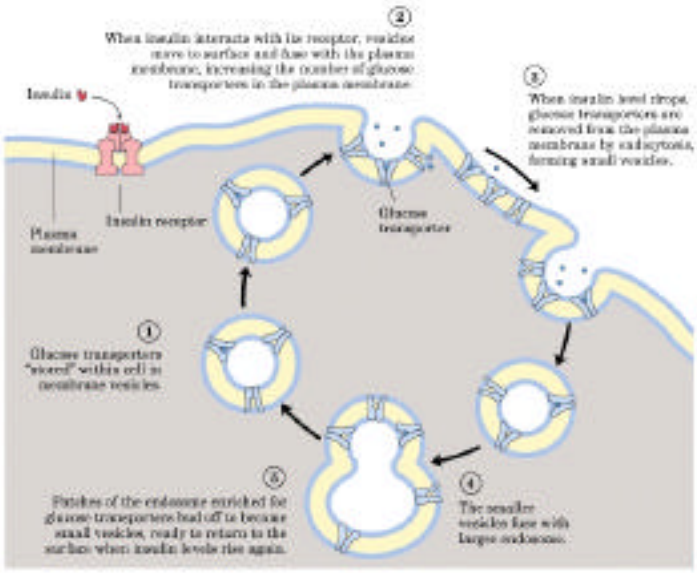

Fig. 34. Regulation by insulin of glucose into myocyte.

Type II. The water permeability of epithelial cells lining the renal collecting duct in the kidney is due to the presence of an aquaporin (AQ-2) in their apical plasma membranes (facing lumen of the duct). Antidiuretic hormone (also called vasopressin) regulates the retention of water by mobilizing AQP-2 molecules stored in vesicle membranes within the epithelial cells, much as insulin mobilizes GluT4 in muscle and adipose tissue. When the vesicles fuse with the epithelial cell plasma membrane, water permeability increases dramatically and more water is reabsorbed from the collecting duct and returned to the blood. When ADH level drops, AQP-2 is resequestered within vesicles, reducing water retention. In the relatively rare disease diabetes insipidus, a genetic defect in Aqp-2 leads to impaired water reabsorption by the kidney. The result is excretion of copious volumes of very dilute urine.

Selected Techniques in Membrane Transport:

Detection methods

Since membrane transporters do not chemically modify or alter its transport substrates, transport activity can only be measured by monitoring the change in substrate concentration on the either side of the membrane. This requires that the substrate molecules deliver some sort of a signal that can be detected by biophysical or biochemical means.

Direct measure

Fluorescent substrates

Fluorescent molecules absorb light at one wavelength (the excitation wavelength) and emits light (fluoresces) at a specific and longer wavelength. Most fluorescent molecules

emit visible light, and the emitted light can be detected and measured by fluorescent spectroscopy.

Radioactive substrates

Radioisotopes or radioactive analogs of substrates offer the highest sensitivity and in most cases are the only tools available to study transport phenomena. Quantitative analysis, like determination of K_m and V_{max} can be efficiently determined using radioactive compounds.

Indirect measure

Fluorescent probe

Fluorescent probes that show a spectral response upon binding to specific ions have enabled researchers to investigate changes in intracellular ion concentrations using fluorescence microscopy, flow cytometry and fluorescence spectroscopy.
Examples: Derivatives of the Ca^{2+} chelators EGTA, Fura-2 pentapotassium salt.

Measuring ion currents by Patch-clamping

Ion channel function can be measured electrically: A single ion channel typically remains open for only a few milliseconds, beyond the limit of most biochemical measurement. Ion fluxes must therefore be measured electrically, either as charges in V_m (in millivolt range) or as electrical currents I (in microampere or picoampere), using microelectrodes and appropriate amplifiers. Patch-clamping, a technique in which very small currents are measured through a tiny region of the membrane surface containing only one or a few ion channel molecules, reveals that as many as 10^4 ions can move through a single ion channel in one millisecond. This represents a huge amplification of the initial signal; for the acetylcholine receptor, for example, the signal may have been only a few molecules of acetylcholine (Fig. 35).

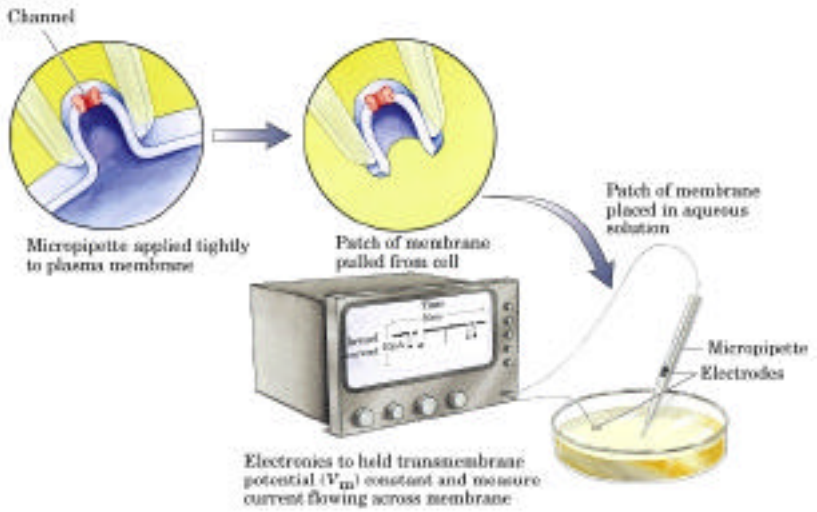

Fig. 35. Electrical measurement of ion channel function by Patch-clamping.

Experimental systems

Intact cells

Accumulation assay

For inwardly directed transport activity, the rate of transport and steady-state accumulation of the substrate can be studied in intact cells. Mammalian cells that grow attached to the substratum are easy to use, since nonspecific binding and extracellular substrate molecules can be removed by simply changing the medium. For free floating cells, a rapid filtration or centrifugation technique has to be used for separating the cells from the free substrate and for removing nonspecific binding.

Efflux assay

For outwardly directed transport, cells could be loaded with the substrate first, either in the presence of a specific inhibitor of the transporter or by metabolic starvation. Following loading, cells should be washed and removed to a substrate- and/or inhibitor - free medium with exogenous energy source, such as glucose. The rate by which the intracellular substrate concentration drops gives an indirect measure of the transport activity (rate of efflux).

De-energization

Energy-dependent transporters that rely on chemical or electrochemical energy to drive transport can be inactivated by metabolic starvation of the cells with agents like 2-deoxyglucose and sodium azide (NaN_3). These agents also help loading the cells with substrate molecules prior to an efflux assay.

Inside-out vesicles (Fig. 36)

Studies in membrane vesicles have been central in the development of new concepts, in understanding membrane transport, and in characterizing transport systems. Vesicles can be prepared from native or reconstituted membranes. Marker enzyme assays are used to evaluate the membrane preparation/purification in terms of yield, enrichment, subcellular fractionation, and orientation (sidedness) of the vesicles. Essentially, the vesicle transport assay involves incubation of membrane vesicles with radiolabeled substrate for a period short enough to reflect the initial transport velocity. At the end of the desired time period, further transport is blocked and vesicles are collected by rapid filtration through 0.2-0.45µm pore-size filter papers. Inside-out membrane vesicles are especially useful for studying membrane transporters that are responsible for solute transport out of the cell. In inside-out membrane vesicles the orientation of the transport protein will be such that transport activity can be studied by measuring direct accumulation of the substrate within the vesicle. Thus a positive signal is associated with the function of the transporter. In this assay, the substrate molecule should not be readily permeable through the lipid bilayer. Also a considerable amount of proteins should be expressed in the cells from which vesicles are prepared.

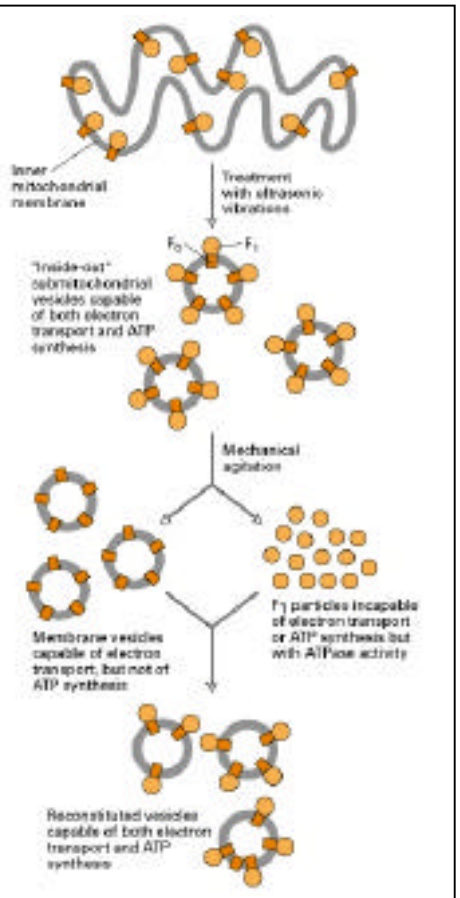

Fig. 36. Reconstitution of F_0F_1 in inside-out membrane vesicles.

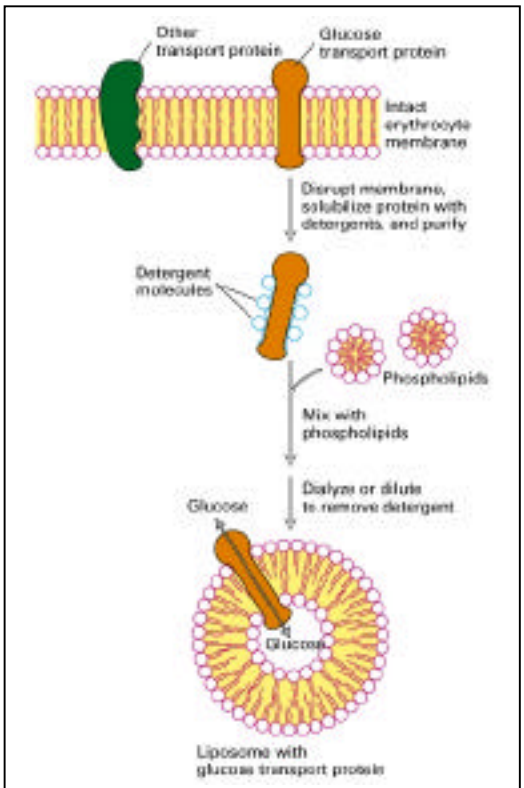

Fig. 37. Reconstitution of a membrane transporter into proteoliposomes

Purified and reconstituted protein (Fig. 37)

Purification of transport proteins from membrane preparations permits extensive biochemical analysis of the transporter. Reconstitution of the protein fraction into liposomes allows investigators to perform functional assays of transport activity in order to evaluate the purification. The first step in purification of transport activity involves solubilization of membrane preparations using detergents. The solubilized protein is then purified and reconstituted into artificial phospholipid membranes. Several purification-reconstitution studies have offered insight into the structures and functions of transport proteins. The nature of the detergent and the composition of the lipid mixture used in this technique are important determinants in preserving the activity of the transporter.

table 12–4

Four Classes of Transport ATPases

	Organism or tissue	Type of membrane	Role of ATPase
P-type ATPases			
Na^+K^+	Animal tissues	Plasma	Maintains low $[Na^+]$, high $[K^+]$ inside cell; creates transmembrane electrical potential
H^+K^+	Acid-secreting (parietal) cells of mammals	Plasma	Acidifies contents of stomach
H^+	Fungi (*Neurospora*)	Plasma	Create H^+ gradient to drive secondary transport of extracellular solutes into cell
H^+	Higher plants	Plasma	
Ca^{2+}	Animal tissues	Plasma	Maintains low $[Ca^{2+}]$ in cytosol
Ca^{2+}	Myocytes of animals	Sarcoplasmic reticulum (endoplasmic reticulum)	Sequesters intracellular Ca^{2+}, keeping cytosolic $[Ca^{2+}]$ low
Cd^{2+}, Hg^{2+}, Cu^{2+}	Bacteria	Plasma	Pumps heavy metal ions out of cell
V-type ATPases			
H^+	Animals	Lysosomal, endosomal, secretory vesicles	Create low pH in compartment, activating proteases and other hydrolytic enzymes
H^+	Higher plants	Vacuolar	
H^+	Fungi	Vacuolar	
F-type ATPases			
H^+	Eukaryotes	Inner mitochondrial	Catalyze formation of ATP from ADP + P_i
H^+	Higher plants	Thylakoid	
H^+	Prokaryotes	Plasma	
Multidrug transporter			
	Animal tumor cells	Plasma	Removes a wide variety of hydrophobic natural products and synthetic drugs from cytosol, including vinblastine, doxorubicin, actinomycin D, mitomycin, taxol, colchicine, and puromycin

Table 2. Types of transport ATPase.

Note: The handout is prepared from materials covered in the following books.

1) ***Lehninger Principles of Biochemistry*;** David L. Nelson and Micdhael M. Cox, 2000. (Worth Publishers)
2) ***Molecular Cell Biology***; Harvey Lodish, Arnold Berk, S. Lawrence Zipursky, Paul Matsudaira, David Baltimore and James Darnell, 2000. (W. H. Freeman and Company)
3) ***Membrane Transport***; Lon J. Van Winkle, 1999. (Academic Press)
4) ***Molecular Biology of the Cell***; Bruce Alberts, Dennis Bray, Julian Lewis, Martin Raff, Keith Roberts and James D. Watson, 1994. (Garland Publishing Inc.)

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